236. Eiichi Takagi and Mitsuo Mangyo: Studies on Reactions between Aromatic Aldehydes and α-Amino Acids. V. Results obtained by Paper Chromatography. (1).

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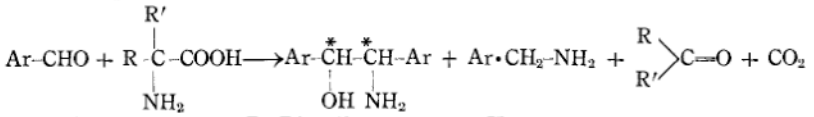
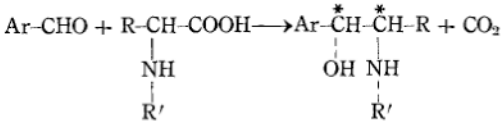
In the preceding paper of this series[1~4] it has been shown that there are differences of types in the reaction of aromatic aldehydes with α-amino acids, according to the form of nitrogen atom of the latter. The main reaction types are as follows:

1. Amino acids with primary amino group (Reaction of Type 1).
(New reaction reported in the earlier work[1~4].)



(Ar=aryl group; R, R′=alkyl group or H)

2. Amino acids with secondary amino group[5] (Reaction of Type 2).
(Akabori's reaction)



(Ar=aryl group; R=alkyl group or H; R′=alkyl group)

3. Amino acids with tertiary amino group[3].
No reaction was observed.

The present paper gives further evidences obtained by paper chromatography in these reactions. Despite the fact that reaction mixture of Type 1 (see Table II) gave four spots, two of which developed yellow color at first and then after few hours changed to purple, and the other two which exihibited purple color directly with ninhydrin in the usual manner, reaction mixture of Type 2 gave only two spots, which exhibited purple color directly without change. In the studies with authentic specimens, it was elucidated that the spots which showed color change belonged to 1,2-diphenyl ethanolamine or benzylamine types of amines.

Although, in the preceding papers[1~4], diastereoisomers were separated, in present studies by paper chromatography we could not determine them separately. In the examinations with diastereoisomers of ephedrine and 1,2-diphenyl ethanolamines the same results were found.

Reactions of Type 1 were studied using benzaldehyde and piperonal as aromatic aldehydes, glycine, alanine and α-aminoisobutyric acid as α-amino acids, and reactions of Type 2 with the same aldehydes and *N*-methylalanine (see Table I). The results are shown in Table II and Fig. 1, in which A(A′), B(B′), C(C′) and D(D′) show the kinds of spots. It was assumed from their Rf values and colors developed that spots A(A′) (Y→P) corresponded to 1,2-diphenyl ethanolamine types of amines, B(B′) (Y→P) to benzylamine types of amines, C(C′)(P) to ephedrine types of amines, and D(D′) to lower aliphatic amines, respectively. It is proved that the amines of type A(A′) and B(B′)

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1) E. Takagi: This Journal, **71**, 648 (1951).
2) E. Takagi, H. Ichikawa, J. Ensaka: Ibid., **71**, 652 (1951).
3) E. Takagi: Ibid. **71**, 655 (1951). 4) E. Takagi: Ibid., **71**, 658 (1951).
5) S. Akabori, K. Momotani: J. Chem. Soc. Japan, **64**, 608 (1943); C.A., **41**, 3774 (1947).

are essential to the reaction of Type 1, being independent of the structure of α-amino acids. The spot D in the reaction No. 1 may be due to methylamine, because Curtius and Lederer[6] reported its formation. In the case of benzaldehyde and alanine, the reaction Nos. 2 and 3, spots C may be due to nor-ephedrine, because its formation has been reported by Akabori and Momotani (*loc. cit*). The special color development of the above-mentioned types of amines are probably due to their specific constitution,

$$\mathrm{Ar{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}{-}R}\ (\mathrm{Ar}=\text{Aryl group};\ \mathrm{R}=\mathrm{H}\ \text{or}\ \mathrm{Ar{-}\underset{\displaystyle OH}{\underset{|}{CH}}{-}}).$$

The special color development of benzylamine types of amines is known in literature[7].

Further investigations are now in progress in this laboratory.

Experimental

Reactions.—1/200 Mole of α-amino acid and 3/200 mole of aromatic aldehyde are mixed and heated to reaction temperature (see Table I) in an oil bath. The reactions examined are given in Table I.

Table I.

Reaction No.	Aldehyde	α-Amino Acid	Reation Condition: Temp., °C	Reation Condition: Time (min.)	Amines isolated in preceding papers
1	Benzaldehyde	Glycine[a]	150~160°	60	*dl*-1,2-Diphenyl ethanolamine, and its iso form, benzylamine, methylamine (*loc. cit*)
2	Benzaldehyde	*dl*-Alanine[a]	150~160°	40	*dl*-1,2-Diphenyl ethanolamine, and iso form, benzylamine.
3	Benzaldehyde	*dl*-Alanine[a]	*150~160°	40	*dl*-1,2-Diphenyl ethanolamine and its iso form, benzylamine, nor-ψ-epedrine[5].
4	Benzaldehyde	*dl*-α-Aminoisobutyric acid[a]	150~160°	40	1,2-Diphenyl ethanolamine and its iso form, benzylamine.
5	Benzaldehyde	*dl*-*N*-Methylalanine[b]	150~160°	40	*dl*-Ephedrine, *dl*-ψ-ephedrine.
6	Piperonal	Glycine[a]	160~170°	60	Piperonylamine, 1,2-di-(3,4-methylenedioxyphenyl) ethanolamine.
7	Piperonal	*dl*-Alanine[a]	160~170°	60	
8	Piperonal	*dl*-Alanine[a]	*150~160°	40	
9	Piperonal	*dl*-α-Aminoisobutyric acid[a]	160~170°	60	
10	Piperonal	*dl*-*N*-Methylalanine[b]	160~170°	40	1,-(3,4-Methylenedioxyphenyl)-2-methylamine propan-1-ol, (two diastereoisomers).

a) Reaction of Type 1.
b) Reaction of Type 2.
* 2 cc. of pyridine was used as solvent.

Paper Chromatography.—The authors carried out paper chromatography with following details.
Sample: Reaction mixture in 2 cc. ethanol solution. No addition of ethanol when pyridine is used as the solvent.
Developing method: Usual descending method.
Filter Paper: Toyo Roshi No. 2, 2 cm × 40 cm.
Developing agent: Mixture of butanol, acetic acid and water (4:1:1).
Developing time: 10 to 16 hours.
Chromatograms obtained by ninhydrin solution in butanol are shown in Fig. I.

6) Curtius, Lederer: Ber., **19**, 2462 (1886).
7) K. Satake: "Chromatograph", p. 88, (Kyoritsu Zensho 1951).

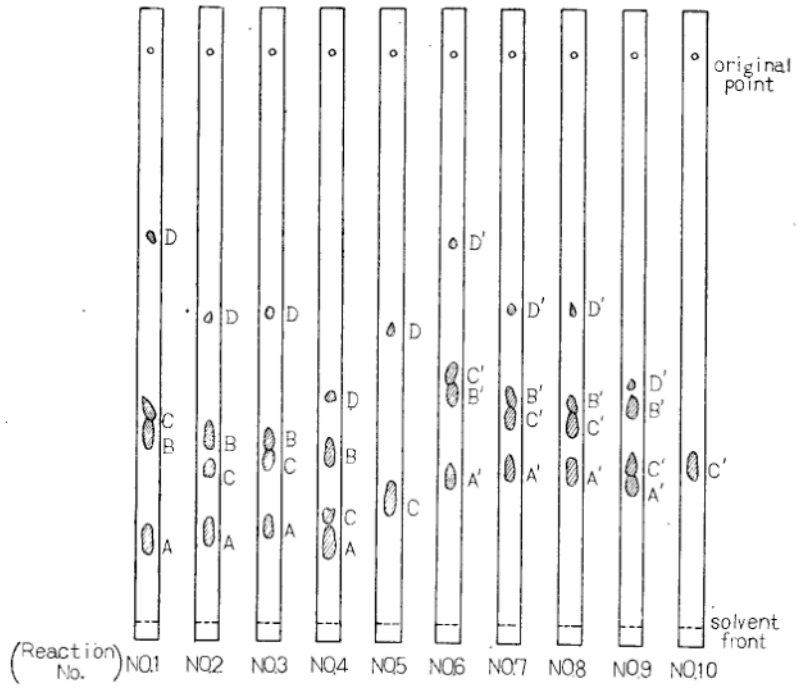

Fig. 1

Rf values of the spots and colors developed are given in Table II.

Table II.

Reaction No.	Color (Y→P)			(P)		Reaction No.	Color (Y→P)			(P)		
	Spot	A	B	C	D		Spot	A′	B′	C′	D′	
1		.84	.68	.63	.33	6		.74	.60	.56	.33	New reaction
2		.84	.67	.73	.47	7		.73	.59	.64	.45	
3		.84	.68	.72	.46	8		.73	.61	.65	.45	
4		.86	.70	.81	.60	9		.75	.61	.72	.58	
5		—	—	.78	.47	10		—	—	.72	—	Akabori's reaction

Y=yellow ; P=purple

A(A′)=Spots of 1,2-diphenylethanolamine-type of amines. } Independent of the structure of α-amino acids.
B(B′)=Spots benzylamine-type of amines. }
C(C′)=Spots of epedrine-type of amines. } Dependent on the structure of α-amino acids.
D(D′)=Spots assumed to be of lower aliphatic amines. }

Although in the preceding paper diastereoisomers of 1,2-diphenyl ethanolamine, ephedrine and 1-(3,4-methylenedioxyphenyl)-2-methylaminopropan-1-ol were separated from reaction mixtures, in present experiments the diastereoisomers were not identified separately. In preliminary experiment the mixture of *dl*-ephedrine and *dl*-ψ-ephedrine gave only one spot with the same experimental condition of the control mentioned in the later paragraph. The same results were obtained with the diasteroisomers of 1,2-diphenylethanolamines.

In the case of the reaction of Type 2, Akabori's reaction, only two spots of purple color were observed, one due to ephedrine-type of amines and the other probably of lower aliphatic amines. The spots A(A′) or B(B′) are not observed in this reaction. Therefore, the reaction of Type 1, the new reaction, is distinctly different from the reaction of Type 2, Akabori's and more complex than the latter.

Control with authentic specimens—Several amines obtained in the preceding work were used for the control: 1,2-diphenyl ethanolamine, 1,2-di-(3,4-methylenedioxyphenyl) ethanolamine, 1-(3,

4-methylenedioxyphenyl) 2-methylaminopropan-1-ol, ephedrine, ψ-ephedrine, benzylamine, piperonylamine, and benzal-1,2-isodiphenyl ethanolamine.

Commercial methylamine, ethylamine and benzal-1,2-isodiphenyl ethanolamine from Erlenmeyer's method[8] were used also for the control. These amine hydrochlorides were used as 1% aqueous solutions and examined under the same conditions as the reaction mixtures by paper chromatography. Rf values and color developed are given in Table III.

Table III.

Amine	Rf value	Color with ninhydrin
dl-1,2-Diphenylethanolamine[a]	.87	yellow → purple
dl-1,2-Di(3,4-methylenedioxyphenyl)ethanolamine.[a]	.75	yellow → purple
dl-1-(3,4-Methylenedioxyphenyl)-2-methylaminopropan-1-ol[a]	.73	purple
dl-Ephedrine[a]	.77	purple
dl-ψ-Ephedrine[a]	.76	purple
Benzylamine[a]	.69	yellow → purple
Piperonylamine[a]	.60	yellow → purple
Isopropyl amine[9]	.57	purple
Ethylamine	.47	purple
Methylamine	.32	purple
Benzal-1,2-isodiphenyl ethanolamine[a]	.85	yellow → purple
Benzal-1,2-isodiphenyl ethanolamine[b]	.87	yellow → purple

a) Specimen from previous work (*loc. cit.*)
b) Specimen from Erlenmeyer's method[8].

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Summary

On the reactions of aromatic aldehydes and α-amino acids[1~5], further evidences were obtained by paper chromatography. It is proved that the reaction of Type 1 gave four color spots of amines with ninhydrin, two yellow spots, which changed to purple after a few hours, and two purple spots, whereas the reaction of Type 2 gave two purple spots. The four spots of the former reaction were assumed to correspond to the types of amines: type A(A′) (independent of the structure of α-amino acids), 1,2-diphenyl ethanolamine-type (Y→P); type B(B′) (independent of the structure of α-amino acids), benzylamine-type (Y→P), type C(C′) (dependent on the structure of α-amino acids), norephedrine-type (P), and type D(D′) (dependent on the structure of α-amino acids), lower aliphatic amines (P), respectively. The two spots of the latter reaction were assumed to correspond to the types of amines: type C(C′), ephedrine-type (P), and type D(D′), lower aliphatic amines (P), respectively. It is proved that the amines of type A(A′) and B(B′) are essential to the reaction of Type 1.

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8) Erlenmeyer, Jun.,: Ann., **284**, 36 (1895); **307**, 85, 134 (1899); **337**, 222 (1904).
9) J. M. Bremner, A. H. Kenten: Biochem. J., (1951) 653.